

CLAIMS

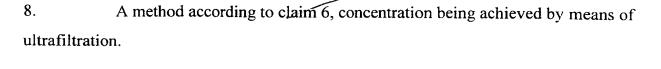
1. A bacterial autoinducer, characterised in that it has substantially the following properties:

- i) it is produced in response to noradrenaline in serum SAPI medium;
- ii) it is heat stable;
- iii) it is stable to lyophilisation;
- iv) it has a negative charge;
- v) it is polar;
- vi) it is hydrophilic;
- vii) it will not partition into organic solvents;
- viii) it is capable of binding positively charged metal ions; and
- ix) it has a molecular weight of about 300-1500 daltons
- 2. A bacterial autoinducer according to claim 1, further characterised in having at least one of the following characteristics:
 - i) it has absorbtion maxima at 255,325 and 500-550nm; and
 - ii) it is stable in prolon/ged storage in a dried state and/or in solution.

3. A bacterial autoinducer according to either one of claims 1 or 2, further characterised in having at least one of the following characteristics:

- it is produced in substantially smaller quantities by bacteria grown in LURIA broth, Tryptone soya broth, M9 minimal medium and Davis-Mingioli/minimal medium than by the same bacteria grown in serum SAPI/medium;
- ii) it has a reddish-pink colour, reversibly decolorisable by reducing the pH to <4;
- iii) it contains sérine;
- iv) its synthesis involves the entA and entB gene products;

- v) its synthesis is not stimulated by conditions of Fe starvation;
- vi) it is synthesised in conditions of excess Fe;
- vii) its entry into bacteria/occurs via a tonB dependent receptor;
- viii) it is inactivated by oxidation;
- ix) it is inactivated by extreme pH; and
- x) it is resistant to degradation by ribonuclease, deoxyribonuclease, trypsin, pepsin, V8 protease, proteinase K, acid phosphatases, alkaline phosphates and phosphodiesterase.
- 4. A bacterial autoinducer according toward one of the preceding claims, being an E.coli, Hafnia alvei or Salmonella autoinducer
- 5. A method for isolating and purifying a bacterial autoinducer, comprising the steps of:
 - i) collecting a sample containing the autoinducer;
 - ii) fractionating the sample to isolate fractions corresponding to molecular weights of approximately 300-1500 Daltons; and
 - eluting the isolate of (ii) on an anion-exchange chromatographic column and selecting the fraction containing the autoinducer.
- 6. A method according to claim 5, comprising the additional step of concentrating the sample prior to fractionating.
- 7. A method according to claim 6, concentration being achieved by passing the sample through an approximately $0.2 \mu m$ diameter filter, lyophilising the sample and passing it through an approximately $0.2 \mu m$ diameter filter.



9. A method according to claim 8, ultrafiltration being performed with a molecular weight cut-off of approximately 100 Daltons.

A method according to any one of claims-5=9, the sample being collected from a culture containing bacteria and the autoinducer.

- 11. A method according to claim 10, the sample being a supernatant collected from a centrifuged culture containing bacteria and the autoinducer.
- 12. A method according to any one of claims 5-11, size exclusion gel filtration being performed using a buffer of approximately 100 mM ammonium bicarbonate, pH 8.0, anion exchange purification being performed on an anion exchange column and triethylammonium bicarbonate.
- 13. A method according to any one of claims 5-11, size exclusion gel filtration being performed using a buffer of approximately 20 mM potassium phosphate containing 150 mM NaCl, pH 7.4, anion exchange purification being performed on an anion exchange column and NaCl gradient.
- 14. A method according to any one of claims 5-13, the bacterium from which the autoinducer is derived being *E. coli*, Salmonella or Hafnia alvei.
- 15. A bacterial autoinducer isolated and purified according to the method of any one of claims 5-14.

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16. The use of a bacterial autoinducer according to any one of the preceding claims in a method of inducing bacterial growth, the production of bacterial toxins or the production of bacterial adhesins.

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